2000

Center of Excellence Five Year Report, 1995-2000

College of Veterinary Medicine

CENTER OF EXCELLENCE IN LIVESTOCK DISEASES AND HUMAN HEALTH

FIVE YEAR REPORT 1995-2000

COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF TENNESSEE, KNOXVILLE

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CENTER OF EXCELLENCE FOR LIVESTOCK DISEASES AND HUMAN HEALTH
COLLEGE OF VETERINARY MEDICINE
UNIVERSITY OF TENNESSEE

The Center of Excellence for Livestock Diseases and Human Health has had a significant role in addressing the needs of animal agriculture in Tennessee and in providing a unique dimension to human health research. The College of Veterinary Medicine has been a true pioneer in the use of animal models to investigate several animal diseases and recently has provided leadership in using laboratory models and thereby reducing the number of animals used in research. It is unlikely that significant progress in the understanding of these diseases could have been made if these animal and laboratory models had not been available. The mission of this Center of Excellence is indeed very important. Within the broad research definition of the Center, the following five areas have been emphasized:

Host Defense
Infectious diseases/population medicine
Molecular genetics and carcinogenesis
Reproduction
Toxicology

The center has established the following goals:

1. To improve the quality of human life by improving animal health.
2. To augment livestock disease research capabilities in the Institute of Agriculture.
3. To identify and characterize laboratory and animal models of important human diseases.
4. To study animal/laboratory models for better understanding of human health.
5. To study the mechanisms of disease development and characterize causative agents of common diseases important to the State of Tennessee.
6. To improve the capabilities of the College of Veterinary Medicine, the College of Agricultural Sciences and Natural Resources and the Agricultural Experiment Station to manage these diseases.
7. To improve the facilities to enable the College of Veterinary Medicine to study more effectively infectious and toxic diseases of animals.
8. To disseminate through the Extension Service practical information required to reduce the incidence of livestock diseases.
9. To develop new strategies for the prevention of disease.
10. To improve facilities and expertise to enhance research training.
11. To develop innovative approaches to the treatment of human diseases.

The Center of Excellence has made a significant contribution to each of these goals and are documened in this "Program Report" and in the description of "Core Laboratory" activities. Performance metrics with respect to scientific publications, presentations and

extramural funding have exceeded benchmarks to impressive degrees (see tables on benchmarks and funding). In the most recent fiscal year, 1999-2000, the return of the State's investment in the Center was almost six fold, a record for the Center (see "Funding Levels"). The number of scientific communications in the form of refereed publications by Center-based investigators for the reporting period 1995 – 2000 was excellent (an average of 7.9 per participant per year).

Research results have been disseminated to fellow scientists in prestigious, refereed international journals and to stakeholders in popular journals and magazines distributed regionally and nationally. Numerous invitations have been extended to many faculty in the Center to make keynote/plenary presentations at national/international scientific meetings. They include Drs. D.A. Brian, B.T. Rouse, J. Oliver, S. Oliver, H.M. Schuller and T.W. Schultz.

This report features the accomplishments and activities of investigators and research teams that constitute the core of the Center. Their achievements serve a crucial function in promoting the College of Veterinary Medicine, the Institute for Agriculture and the University of Tennessee. Furthermore, they continue to ensure that the Center maintains its strong competitive position and contribute to the fiscal health of our research environment. The report endeavors to emphasize the strength of the Center and give a clear indication of how it meets its objectives.

Accomplishments

The accomplishments and activities of "Core Laboratories" in the Center are summarized in this report in a manner that can be appreciated by a wide readership. Accomplishments can be measured in a variety of ways and include peer-reviewed scientific publications, leverage of the Center in attracting appropriate extramural funding, the number of national/international presentations by participants, effective dissemination of discovery in the Center, the number of scientists brought to the University to foster scientific collaboration/communication, training of scientists and other benchmarks (see tables on funding and benchmarks). Center of Excellence faculty published an impressive 728 peer-reviewed scientific articles over the reporting period of 1995-2000. This represents an average of 7.9 per year per participant, a truly remarkable achievement. Furthermore, these faculty made 326 presentations at national/international scientific meetings over the reporting period. Please see the discussions on "Funding Levels", "Research Funding", "Research Training", "Culture for Discover", "Dissemination of Research to the General Public", "Personnel Changes" and details on the activities in "Core Laboratories" for more information on achievements.

Research Funding

The "core" members of the Center of Excellence are Drs. Barry Rouse, Hildegard Schuller, David Brian, Terry Schultz, Jim Godkin, Steve Oliver, Jack Oliver, Xuemin Xu and Hwa-Chain Wang. Included among this group are several pre-eminent scientists with international reputations for excellence. They include Drs. Rouse, Schuller, Brian, Jack Oliver, Steve Oliver and Schultz. These investigators are established researchers and have a superior record of extramural funding and scholarship that spans over two

decades. The core members contribute most of the benchmarks of the Center and have an important leadership role in the program.

An important goal of the Center of Excellence in Livestock Diseases and Human Health is to support researchers and to promote research by a variety of mechanisms. State fiscal restraints for several years have restricted our ability to recruit and hire competitive researchers. The Center has had a significant impact over the last two years in recruitment of researchers with ongoing research programs. The existence of the Center and its ability to contribute to start-up packages has made the difference in these recruitment efforts.

The Center of Excellence emphasizes the following six specific areas: Infectious Diseases/Population Medicine, Toxicology, Reproduction, Host Defense and Molecular Genetics and Carcinogenesis. Each participant contributes to at least one of these areas of emphasis. The Center's underlying philosophy is to enhance the capacity of young or new investigators to compete for extramural funding and to assist established researchers in maintaining extramural support. The Center does not serve as a primary source of research funding for faculty. The main criteria used for funding proposals include scientific merit, likelihood of leading to extramural funding and relevance to the Center's objectives. Proposals submitted to the Center for funding are reviewed by the Research and Graduate Programs Advisory Committee. The latter has one representative from every department of the College of Veterinary Medicine and has been chaired by Dr. Hildegard Schuller. Requests for 89 projects were funded during the reporting period from 1995 to 2000. The following projects were supported this year by the Center:

CENTER OF EXCELLENCE PROPOSALS FUNDED IN 2000

Name	Title of Proposal
Andrews, Frank	Pathogenesis of acid injury in the non-glandular region of the equine stomach
Brian, David	Genetic structural elements regulating RNA synthesis during coronavirus replication
Cui, Mei-Zhen	Mechanisms of lipoprotein induction of tissue factor gene expression in smooth muscle cells
Godkin, James	Retinoids in oocyte maturation and embryonic development
Mathew, Alan	Characterization of bacterial resistance elements in swine herds
McEntee, Michael	Cellular mechanisms of NSAID-mediated regression in intestinal tumors
New, John	A pilot project to survey rodent populations in the Great Smoky Mountains National Park for the presence of hantavirus and Borrelia species
Oliver, Jack	Studies of tall fescue toxin induced aminoacidemia in cattle
Oliver, Steve	Identification and characterization of streptococcal virulence factors
Rouse, Barry	Role of chaperone bound peptides in the induction of antiviral CTL responses
Schuller, Hildegard	Effects of NNK on beta adrenergic growth regulation of pulmonary adenocarcinoma in vitro
Schultz, Terry	Quantification of the underestimation of toxic potency in microscale testing
Tithof, Patricia	Effects of components of cigarette smoke on endothelial cell and vascular smooth muscle cell arachidonic acid metabolism and apoptosis
Wang, Hwa-Chain	Novel intracellular signaling pathways leading to cell quiescence and apoptosis

Additionally, COE funds were distributed to Drs. Xuemin Xu and Hwa Chain Wang as part of a "start-up" package approved during recruitment of these investigators.

Equipment and Facilities

Annually, proposals for equipment purchases are solicited from eligible faculty. Criteria considered in the allocation of these funds included justification of need, equipment availability in adjacent laboratories, and the number of investigators who may benefit. The latter is of particular importance; i.e. whether these pieces of equipment could be used in a "core" role benefiting as many researchers as possible. Sixty eight requests for purchase or upgrade of 98 pieces of equipment were either fully or partially funded by the Center over the reporting period.

Requests from 14 investigators for 18 pieces of equipment were funded by the Center of Excellence this past year. Researchers benefiting from the Center grants were Drs. Henry S. Adair, Joseph Bartges, David Brian, Dennis Geiser, James Godkin, Alan Mathew, Jack Oliver, Stephen Oliver, Barry Rouse, Hildegard Schuller, Terry Schultz, Carla Sommardahl and Hwa-Chain Wang.

Renovations funded by the Center of Excellence over the past five years included extensive repair and renovation of walk-in coolers in A329A and A307, laboratories in the Veterinary Teaching Hospital. These coolers are used primarily by investigators participating in the COE. Venting problems with class II type B2 and class II type A hoods in A307A laboratory and in the P3 biosecurity facility required extensive remodeling and certification. Similarly, repair and maintenance of several pieces of research equipment was supported by COE funds. This included an ultracentrifuge and the transmission electron microscope. The Johnson Biotechnology Animal Science facility was completed and provides state-of-the-art laboratories and animal holding facilities for some of our investigators. This facility includes two large laboratory animal rooms and a student teaching area and consequently some space has become available in the Veterinary Teaching Hospital for research activities. An addition to the College of Veterinary Medicine also was completed during this reporting period. It houses the Office of Laboratory Animal Care and supporting staff. These activities impact the research program and occupation of these facilities has provided relief for housing graduate students and post-doctoral fellows in space previously occupied by this office.

With support from the Center of Excellence, Dr. Hildegard Schuller and other COE participants in 1999 secured a substantial equipment grant from NIH. This grant supported the purchase of a Facscan-Cell Sorter which will be used for basic and clinical research. Matching funds provided by the Center was critical in the success of this application. It will greatly enhance the research capacity of the Center.

Research Training

The College of Veterinary Medicine funds at least 10 positions for Ph.D. training of students with a professional medical degree. Some of these positions are based in the Department of Pathology (as part of their residency/Ph.D. program) and some are awarded without restriction. Most of these students become linked with Center of Excellence faculty. These young investigators significantly bolster the achievements of the Center. Center faculty that have benefited from these graduate students include Drs. Brian, Tithof, Rouse, Schuller, Hahn, Wilkinson, and Wang. The Center has also provided direct support for graduate students through proposals funded in the annual competition.

In addition, The University of Tennessee's College of Veterinary Medicine is one of the few colleges of veterinary medicine to be chosen as a site for a National Institutes of Health Institutional Training grant. This five-year training grant on the "Molecular and Cellular Pathobiology of Environmental Disease" is funded through the National Institute of Environmental Health Sciences. The grant began in 1995 and is funded through the year 2000; past year expenditures were $218,931. This training grant is centered in the Department of Pathology, but also involves scientists from other departments as well as important collaborators in the Life Sciences Division at the Oak Ridge National Laboratory. Dr. David O. Slauson has been Program Director for the grant and Pathology Department Head. The research training sponsored by this NIH grant emphasizes the basic molecular and cellular biology of disease, including environmental disease. The existence of the Center of Excellence in the College was a crucial element in the consideration by NIH for this award. Three DVM graduate students were supported last year by these NIH funds and worked in the laboratories of COE-associated scientists. They are Drs. Brian Jull, Steven Grubbs and Sharon Witonsky. A total of 11 students have benefited from the program over the last five years.

Student Awards

An important mechanism by which the Center of Excellence promotes biomedical research is to provide summer opportunities for veterinary students to do investigational work in research laboratories in the College of Veterinary Medicine. This past year the Center funded eight requests from first- and second-year students. The students are required to provide a summary of their work, which then is entered into a competition judged by Phi Zeta, the veterinary honorary society. This program is very successful. Several students presented their work to faculty and staff of the Institute of Agriculture (see "Culture for Discovery") and at national scientific meetings. Numerous manuscripts detailing results of work done by these students have been submitted for publication to refereed journals. In fact, **over the past five years, this program has resulted in approximately 30 publications in refereed journals, several with the students as senior authors.**

Personnel Changes

Dr. G. Michael Shires, Dean and Director of the Center of Excellence in Livestock Diseases and Human Health retired at the end of the 1999-2000 fiscal year. The new Dean of the College of Veterinary Medicine, Dr. Michael Blackwell, will serve as the new Director of the Center. Because the Dean of the College of Veterinary Medicine has announced the intention of adding an Associate Dean for Research to his administration team, the Assistant Director of the Center likely also will be replaced in 2001. This will represent complete new management of the Center.

Unfortunately, several productive Center participants either retired or resigned during this reporting period, especially from 1995 through 1998. They include Drs. Ted McDonald, Walter Farkas, Mark Miller, Kevin Hahn, Linda Munson, Joyce Merryman and Eric Schultz. Several of these were core members of the Center (McDonald, Farkas, Miller and Hahn). These personnel actions affected our benchmarks, especially

extramural funding levels. However, recent strategic hires have significantly improved the funding status of the Center.

Recent recruitments of faculty with a significant research focus will benefit the Center of Excellence (see discussion on "Funding Levels"). Over the past approximately two years we have recruited seven new researchers who will or are contributing to our Center of Excellence. They include Dr. Hwa Chain Wang (Comparative Medicine Department) who is well funded by NIH and Dr. Patricia Tithoff (Animal Science) who has received very competitive scores for her proposals and likely will be funded by NIH in the future. Dr. Joseph W. Bartges (Department of Small Animal Clinical Sciences) has received substantial funding from a variety of industry sources and the Morris Animal Foundation. Dr. David Slauson, head of the Department of Pathology in 1999, was able to recruit Drs. Xuemin Xu and Mei-Zhen Cui. They brought with them significant research funding from NIH (R01 grants), American Heart Association and foundations. Their research interests also fall within the Center's focus. Dr. Robert Moore, head of the Department of Microbiology, announced the recruitment of Dr. Pam Small from NIH, a well-known expert on tuberculosis. She joined the faculty in October 2000 and plans to do much of her research in the biosafety level three core facility in the veterinary teaching hospital. Tuberculosis is again an emerging disease and Dr. Small's recently received word of substantial funding from NIH to study this organism. Recently, Dr. Hildegard Schuller, Interim Head of the Department of Pathology, announced the approval of a search for an established researcher who would participate in the Center of Excellence of Livestock Diseases and Human Health.

Drs. Dorcas Schaeffer (O'Rourke) and Monica Fann were recruited in 1998 and 2000 to fill the positions of Director and Associate Director of the Office of Laboratory Animal Care respectively. This should have a significant impact in facilitating research at the College of Veterinary Medicine.

Funding Levels

Expenditures out of extramural grants during the reporting period 1995 – 2000 was approximately $11,400,000.00 compared to the States investment of $2,669,910.00 over this period. Extramural support for the Center of Excellence increased dramatically the last year of this five-year period. Retirements and resignations affected its growth for the first two to three years of this five-year period. Therefore funding for the first three years of this reporting period was steady and even declining slightly, but expenditures the past year are the highest ever, surpassing by about $300,000 the amount in 1990-91, the previous record. Our investment in recruitment of researchers the past two to three years now is coming to fruition (see "Personnel Changes"). The State's investment in the Center of Excellence in Livestock Diseases and Human Health remains a healthy one. Extramural funding expenditures this year totaled $3,103,004 whereas the total funding level of all active grants and contracts supporting the Center amounted to $11,946,613. The rate of return of State dollars for the year was 5.95!

Our investigators brought in $2,674,287 of new extramural funds in the past fiscal year (Drs. Godkin, Mathew, McEntee, S.Oliver, Rouse and Schuller – see table 2). Also, I am very encouraged that since the end of the 99-00 fiscal year, several COE faculty appear to have secured new multi-year funding for COE-related research amounting to

approximately $2,605,000. They include Drs. Barry T. Rouse, Michael McEntee and Sharon Patton.

Dr. Schuller and co-workers recently received word that NIH is planning a site visit early in 2001 as the first step of what we hope to be a positive evaluation of a $12,000,000.00 Program Project (PO1) on "Targeting Cell Type-Specific Regulatory Pathways for Lung Cancer Intervention." This is a very encouraging development and it must be noted that the Center has had a role in supporting Dr. Schuller's research for several years now. In fact the Center's emphasis on Molecular Genetics and Carcinogenesis undoubtedly helped set the stage for developing this proposal. Likewise, the Center has also supported Dr. Rouse's program. Dr. Rouse received word recently of funding of a third RO1 NIH grant. Total funding for his research (all years) is $4,598,864. He appears to be the best-funded researcher (at least with respect to NIH funds) at the University of Tennessee.

Culture for Discovery

In the past, the Center promoted a "Research Day" to showcase the achievements of participating faculty and to promote research at the College of Veterinary Medicine. Attendance at this event has declined significantly in recent years. It appears that the format of scheduling an entire day or two for this event was not appropriate for local participation. The past two years the Center has sponsored regular seminars, posters and presentations by participating investigators and students. The program was advertised University-wide and has been very well attended and received. It seeks to foster interest in research and to display the Center's role in promoting research at the Institute of Agriculture.

The College hosted at least 78 visiting research scholars during the reporting period. They were supported by a variety of mechanisms including Center of Excellence funds, the College's "Biomedical Seminar Series" and other funds. These scholars were selected, in part, to contribute to the Center's research emphasis.

VIRAL IMMUNOLOGY LABORATORY

Dr. Barry T. Rouse
Fellows, Staff and Graduate Students: S. Chun, S. Deshpande, S. Kug Eo, U. Kumaraguru, S. Lee, T. Sobhani, M. Zheng

Dr. Rouse's research deals with the recognition and interaction of the body with viral infections. This group has studied herpes simplex virus, an agent that affects the majority of humans. The virus persists indefinitely in infected individuals, and some suffer periodic lesions which are painful and inconvenient. When such lesions occur in the eye, they can lead to blindness. Dr. Rouse's laboratory is involved in studies directed to understand the mechanisms by which herpes simplex infection causes blindness.

Dr. Rouse's approach to understanding the interaction between herpes simplex virus and the immune system is to exploit model infections such as the mouse system as an animal model. Their aim is to understand how cells and molecular events set into play by herpes simplex virus lead to chronic inflammatory lesions or to resolution of disease. Ultimately, it may prove possible to manipulate the host defenses either to achieve protection by vaccines or resolution of injury by substances introduced by gene transfer technology and capable of influencing the immune response.

In the last year they have used mouse models which have been genetically manipulated to cause deficient immune systems and thereby to evaluate whether the ocular lesions are the consequence of an autoinflammatory (self-destructive) reaction. Their results do not support the latter hypothesis. Instead the evidence indicates that herpes simplex replication in the eye causes the output of molecules called cytokines and chemokines which activate certain invading cells of the immune system (T lymphocytes) to release inflammatory substances. This mechanism is referred to as bystander activation. Such a mechanism could represent an important component of any chronic inflammatory reaction.

The other major activity in Dr. Rouse's laboratory is to understand the cell and molecular events that occur during an immune response to novel DNA vaccines. They have identified that a process called "cross priming" is the main event in this process and that it is mediated by a "chaperone molecule" bound to a protein (peptide). They are exploring the use of chaperone-bound peptides as a unique means of vaccination. This could have a major impact in the prevention of viral diseases in people and animals.

The Center of Excellence supports some aspects of this research which is funded primarily by substantial grants from the National Institutes of Health. Their work has generated national and international interest, and the laboratory is recognized as one of the premier viral immunology programs in the country. Dr. Rouse is one of a very select group of investigators in this country holding three R01 NIH awards simultaneously.

MASTITIS RESEARCH LABORATORY

Dr. Stephen P. Oliver.
Fellows and Staff: R. Almeida, W. Fang, B. Gillespie, M. Lewis, D. Luther, S. Ivey, L. Coleman.

Research conducted by Dr. Oliver focuses on mastitis in dairy cows caused by environmental organisms. Several kinds of bacteria are capable of infecting the udder causing mastitis. These pathogens invade the udder, multiply there and produce harmful substances that result in inflammation, reduced milk production and altered milk quality. Control of mastitis is extremely difficult because of the many types and sources of mastitis pathogens that can cause the disease. The National Mastitis Council estimates that mastitis costs U.S. dairy producers over two billion dollars annually. In Tennessee, losses due to mastitis may exceed $25 million annually. Thus, mastitis in dairy cows is likely the most costly disease affecting dairy producers in Tennessee, the U.S., and throughout the world.

Dr. Oliver was the first to show that mastitis in pregnant dairy heifers occurred frequently near calving and that many of these infections persisted into early lactation. His research has resulted in a simple, effective and inexpensive method for controlling mastitis in heifers. Intra-mammary antibiotic infusion before calving, was shown to be an effective procedure for:

1) eliminating many infections in heifers during late gestation
2) reducing the prevalence of mastitis in heifers during early lactation
3) for reducing the prevalence of mastitis in heifers throughout lactation.

He documented that a return of $12-$20 for each dollar spent was possible using this approach.

Several studies over the past 13 years at the UT Dairy Experiment Station involved collection of almost 200,000 milk samples for microbiological evaluation at intervals before calving, during lactation and during the dry period. Data from those studies have been computerized and this mastitis database may be the largest in the world. It now is being exploited for retrospective studies and will provide valuable information on the spread of mastitis pathogens, such as *Streptococcus uberis* and *Streptococcus dysgalactiae,* in high-producing dairy herds. Recently, they evaluated the influence of mastitis on reproduction in Jersey cows and found it profoundly impairs reproduction during early lactation.

Dr. Oliver has been actively seeking the identification of virulence (severity) factors produced by certain mastitis organisms (*Streptococcus* species) and implications of immunity to them. In many dairy herds *Streptococcus uberis* and *Streptococcus dysgalactiae* are responsible for a high proportion of mastitis with varying degrees of severity in lactating and non-lactating dairy cows. Strategies for controlling these mastitis pathogens are poorly defined and inadequate. This research focuses on:

1) genetic characterization of *Streptococcus uberis* and *Streptococcus dysgalactiae*
2) characterization of *Streptococcus uberis* and *Streptococcus dysgalactiae* with particular emphasis on factors involved in adherence and invasion into mammary epithelial cells
3) evaluation of immunity after immunization of dairy cows with components of *Streptococcus uberis* and *Streptococcus dysgalactiae*
4) effectiveness of experimental vaccines to *Streptococcus uberis* and *Streptococcus dysgalactiae* mastitis during the nonlactating period

Dr. Oliver's research group has determined that *Streptococcus uberis* and *Streptococcus dysgalactiae* readily adhered to and invaded cells lining the bovine udder. Chronic infections then may develop, and their intracellular location may protect these bacteria from anti-microbial drugs and host defense mechanisms. Mastitis pathogens cultured in the presence of mammary epithelial (lining) cells in the laboratory synthesize proteins not detected when bacteria are cultured alone. These unique proteins likely are involved in virulence of bacteria, including their capacity to adhere and invade mammary epithelial cells. Thus, culture of mastitis pathogens in the laboratory in the presence of mammary epithelial cells may result in expression of bacterial virulence factors similar to that which occurs in the animal. This important discovery will be exploited for the development of vaccines and management of mastitis.

Dr. Oliver's expertise in mastitis and milk quality has led also to a new research initiative in food safety. The primary goal is to provide comprehensive information on the occurrence and distribution of *Salmonella, Escherichia coli O157:H7,* and *Campylobacter jejuni* in bulk tank milk, feces of cull dairy cows and the environment in dairies. Antibiotic resistance patterns and molecular characterization of foodborne pathogens is also being done.

Dr. Oliver has communicated results of his research via scientific and popular press publications, and via presentations to several different target groups at state, regional, national and international meetings and conferences. In addition, Dr. Oliver has made several presentations to groups such as the University of Tennessee Agricultural Committee Board of Trustees, the Institute of Agriculture Development Board and the 21st Century Campaign Steering Committee and Tennessee Agricultural Experiment Station Department Heads' Conference. Dr. Oliver has given presentations entitled "The University of Tennessee Mastitis Research Program: Making a Difference Through Research" to the Tennessee House of Representativies Agriculture Committee, to The University of Tennessee Institute of Agriculture Alumni Council and to the University of Tennessee Institute of Agriculture Development Board and Agriculture Steering Committee for the 21st Century Campaign.

Dr. Oliver has increased the awareness of scientists, extension specialists, dairy producers and pharmaceutical companies of the importance of environmental pathogens in bovine mastitis. Furthermore, he has discovered fundamentally important information that is critical for controlling the heterogeneous organisms that cause mastitis. Dr. Oliver's research philosophy is to design and conduct innovative and useful studies and to report to a

wide variety of constituents. The ultimate goal of this research is to enable dairy producers in Tennessee, the U.S., and throughout the world to enhance the quantity and quality of milk produced and thus reduce the economic impact of mastitis.

Dr. Oliver's research has been supported for several years by the Center of Excellence, but his primary funding has been derived from substantial grants from foundations, FDA and the pharmaceutical industry.

VIRUS MOLECULAR BIOLOGY LABORATORY

Dr. David A Brian
Fellows and Graduate Students: Dr. S. Senanayake, Dr. K. Nixon, Dr. A. Ozdarendelli, G.D. Williams, S. Raman, C. Gay, H. Wu.

Dr. Brian's interest in basic molecular biology of viruses has resulted in discoveries of a fundamental nature for which his laboratory has received national and international recognition. His research focuses on coronaviruses which cause of some of the most costly respiratory and gastroenteric diseases of livestock and fowl, and disabling diseases of people. Efforts to control coronavirus infections have been frustrated by three major obstacles:

1. An incomplete understanding of how coronaviruses replicate and persist in animals.
2. The ability of coronaviruses to rapidly mutate into new pathogenic variants.
3. The generally weak immune responses in animals to coronavirus vaccination and the logistical problem of inducing protective mucosal (local) immunity in the vulnerable newborn.

The primary research focus in Dr. Brian's laboratory is the molecular biology of coronavirus replication. With funding from the USDA and the NIH, and modest support from the Center of Excellence, they are making an intense effort to understand how five separate genetic elements in the coronavirus function to regulate production of viral proteins and progeny virus. Research is being done also on a sixth genetic region, a hot spot for variability, in an effort to understand the determinants of this process. Genetic recombination (blending) at this site is a mandatory step used by the virus in the generation of messenger molecules that encode portions of the virus' genetic material. It is anticipated that information from these studies will significantly impact the design of new therapeutic strategies.

Of special interest is how a newly discovered element at one end of the virus gene regulates replication of the genetic material. The element is a tRNA-like folded structure (a pseudoknot), that may regulate virus replication by incorporating a cellular protein in the virus replication machinery. Therapeutic interruption of such a virus-protein interaction may lead to a cure of virus infection. Interestingly, one candidate protein in

this interaction is histidyl tRNA synthetase, a factor (autoantigen) in the human disease polymyositis.

Dr. Brian's laboratory has also discovered a small genetic variant of the bovine coronavirus (a viral minigenome) that replicates in the presence of "normal" virus. This minigenome is being experimentally engineered to carry many kinds of potential antiviral molecules into cells. One molecule is an enzyme (a ribozyme) designed to destroy the gene on which the virus depends for replication (the polymerase gene). This novel therapeutic approach would, in theory, cure a virus-infected cell without killing it.

ANTIBIOTIC RESISTANCE LABORATORY

Dr. Alan G. Mathew
Staff and graduate students: R. Clift, S. Chattin, D. Arnett, P. Cullen, P. Ebner, K. Garner, G. Pulliam

Antimicrobial compounds are commonly used in US livestock systems. Therapeutic use of antibiotics continues to play a major role in combating disease organisms, while subtherapeutic use in feeds increases animal performance, decreases the numbers of infectious organisms in the environment, and lowers the prevalence of organisms causing foodborne illness in humans.

In contrast to the above benefits, some evidence suggests that agricultural use of antibiotics may be partly responsible for the emergence of drug-resistant bacteria, which in turn may decrease the efficacy of similar products used in human medicine. However, little information is available on strategies for controlling of antibiotic-resistant organisms. In particular, almost no information is available with respect to modern livestock production facilities, management, environmental conditions, or drug therapies that affect resistance in organisms. Because resistance may be transferred to bacteria from a variety of resistant bacteria and associated hosts, it is important that factors involved are characterized so that more effective control strategies can be formulated.

A primary research focus of Dr. Mathew's group is to characterize genetic factors that lead to antibiotic resistance in animal and human pathogens. They also are investigating how different uses of antibiotics in livestock production affect antibiotic resistance patterns, concentrations, and shedding of foodborne pathogens. They hope to determine the most effective antibiotic therapies and husbandry practices to maintain animal health, while at the same time limiting prevalence of foodborne pathogens and antibiotic resistance of microorganisms in livestock.

Recent work by the group has included a pioneering microbiological survey of swine farms that used or excluded antibiotics in the animals. They determined that pathogenic bacteria from farms that excluded antibiotics were more sensitive to antibiotics. However, resistant isolates occurred on both farm types, and young, recently weaned animals from all farms harbored the greatest number of resistant isolates. This

may indicate that stresses associated with weaning affect bacterial antibiotic resistance patterns, regardless of whether antibiotics were used on the farm.

Dr. Mathew investigated also the effects of antibiotic treatment regimens on resistance patterns of *E. coli* and Salmonella bacteria in pigs. They inoculated pigs with a swine pathogen (K88 *E. coli*) and a foodborne pathogen (*Salmonella typhimurium)* and then treated with aminoglycoside, sulfonamide, and beta lactam antibiotics using various dosing schemes. Dosage methods, dosage levels and rotation with dissimilar antibiotics affected the genesis of resistance.

Another important discovery was that *E. coli* associated with livestock became resistant much more quickly than Salmonella in the same host, possibly indicating a greater ability to acquire resistance elements such as plasmids (R-factors), or a greater ability to generate chromosomal resistance elements. This finding will be very important as we assess the agricultural use of antibiotics and the risks of generating resistance in foodborne pathogens. They now are characterizing the bacterial resistance elements using various DNA-based techniques to determine the relative importance of R-plasmids and chromosomal elements in the acquisition and persistence of antibiotic resistance.

Dr. Mathew is continuing other work to determine how animal stressors such as crowding, low sanitation, and temperature stress affect the prevalence of antibiotic resistance in *Salmonella typhimurium* and *E. coli* in swine. Preliminary data indicate that such stressors increase the percentage of resistant isolates.

Work with Dr. Stephen Oliver has focused on the development of rapid methods for detection and differentiation of Salmonella variants. This will allow us to determine the origin and reservoirs of those bacterial variants that are most responsible for foodborne illness in livestock operations. They have determined that a linked PCR-ELISA assay is capable of rapid (less than 24 hours) differentiation of Salmonella bacteria from livestock samples. Such techniques will be instrumental in the implementation of on-farm Hazard Analysis Critical Control Points (HACCP) strategies for management of specific foodborne pathogens.

Dr. Mathew's research is supported by the Center of Excellence but primary funding for this work is derived from the International Life Science Institute and the National Pork Producers Council.

BIOPHYSIOLOGICAL PATHOGENESIS LABORATORY

Patricia K. Tithof

Collaborators, Staff and Graduate students: Dr. M. Peters-Golden, Dr. H. Schuller, Dr. H.C. Wang, Dr. R. Donnell, M. Elgayyar, M.A. Barnhill

Dr. Tithof's research program in cardiovascular physiology concerns the effects of specific components of cigarette smoke on the biology of blood vessel lining

(endothelial) cells and metabolism of a potent physiological chemical messenger, arachidonic acid. The latter is a fatty acid that is present in high quantities in the membranes of all cells and is a substrate for the production of eicosanoids, a family of biologically active lipid mediators. The latter have an important role in several diseases including asthma, arthritis, cancer and atherosclerosis.

Smoking, which greatly augments the process of atherosclerosis, increases the risk for heart attack or stroke by as much as 50%. Recent epidemiologic studies indicate that a high fish diet or frequent use of aspirin significantly decreases mortality rates due to heart attacks in heavy smokers. Fish contains high levels of certain fatty acids, which decrease the availability of arachidonic acid and also inhibit the production of arachidonic acid-derived eicosanoids. The protective effects of these fatty acids and aspirin against smoking-induced atherosclerosis suggest that components of cigarette smoke stimulate the arachidonic acid pathway. However, no previous studies have focused on the specific components of cigarette smoke responsible for this effect. Several compounds are contained in high concentration within the tar fraction of cigarette smoke. These include methylanthracenes, phenanthrene and benzo(a)pyrene. Benzo(a)pyrene accelerates the development of atherosclerosis in animal models.

Endothelial cells form a single cell layer lining the blood vessel wall. Endothelial cell death and loss is a critical and important event in the early development of atherosclerosis. Loss of the endothelial cell layer results in inflammation of the vessel wall, vasoconstriction and clot formation; events important in the development of heart attacks and strokes. Previous studies indicate that cigarette smoking increases the rate of endothelial cell loss; however, neither the mechanism of cell death nor the specific components of cigarette smoke responsible have been elucidated.

These studies indicate that exposure of porcine aortic endothelial cells or human coronary artery endothelial cells to these compounds in cigarette smoke causes release of arachidonic acid. Furthermore, this release of arachidonic acid is associated with loss of endothelial cell viability through stimulation of a process known as apoptosis or programmed cell death. Apoptosis induced by these compounds can be inhibited by the fatty acid, eicosapentaenoic acid, which exists at high concentrations in fish. These results suggest that methylanthracenes, phenanthrene and benzo(a)pyrene may be important components of cigarette smoke that augment atherosclerosis through a process that involves arachidonic acid-mediated killing of endothelial cells.

Dr. Tithof also has initiated studies to investigate the effects of derivatives of nicotine on endothelial cell function. A derivative of nicotine, NNK, plays an important role in smoking-induced lung cancer. Recent studies in collaboration with Dr. Hildegard Schuller suggest that NNK induces tumor formation by a mechanism that involves release of arachidonic acid by stimulation of certain cell receptors (beta-adrenergic). The finding that NNK binds to beta-adrenergic receptors has important implications concerning a potential effect of NNK on the cardiovascular system. Evidence indicates a role for the beta-adrenergic system in the cause of atherosclerosis because beta-

adrenergic blocking agents prevent endothelial cell injury Beta-adrenergic blockade also reduces endothelial cell injury induced by cigarette smoke.

The effects of NNK on endothelial cell viability were investigated by Dr. Tithof's group. NNK, at very low concentrations caused apoptotic cell death of endothelial cells. Their results suggest that it was the result of NNK binding to beta-adrenergic receptors and that the arachidonic acid cascade was involved.

These studies provide novel findings concerning the effects of specific components of cigarette smoke on endothelial cell function and suggest a novel mechanism by which cigarette smoke augments atherosclerosis. These studies may contribute to the development of effective measures for preventing cardiovascular complications in smokers. Moreover, identifying specific components of cigarette smoke that augment disease may lead to the development of safer cigarettes. These strategies may be particularly important because the incidence of smoking continues to rise, despite intensive efforts to educate people about the hazards of smoking.

REPRODUCTION LABORATORY

Dr. James D. Godkin
Fellows, Staff and Graduate Students: Dr. D. Eberhardt, T. Livingston, H. King, S. MacKenzie, M. Roberts

The focus of Dr. Godkin's research group over the past few years has been the study of proteins that communicate fetal-maternal interactions and result in the successful maintenance of pregnancy. One important discovery was that interferon-tau, a placental protein, interacts with the uterus and alters maternal hormonal balance and maintains early pregnancy in ruminants such as cattle, sheep, goats, and buffalo. Another focus of Dr. Godkin's laboratory is reproductive efficiency with respect to the effect of growth factors and certain vitamin A-like proteins (retinoid-associated) and their genes on the early embryo, ovary, oviduct and uterus.

The major current focus of Dr. Godkin's laboratory is on factors that control development of the early embryo of domestic livestock. Recently, they made the remarkable discovery that treatment of animals with vitamin A-like compounds (retinoids), just prior to ovulation, results in improved viability of embryos that then develop following fertilization. In addition, his laboratory studies indicated that treatment of embryos with retinoids dramatically improved embryonic development. The goals of this research are to improve reproductive efficiency through the use of retinoid administration procedures, to develop more efficient assisted reproductive procedures and to determine the mechanisms by which retinoids affect oocyte (egg) maturation, embryonic development and survival.

This research has the potential to improve reproductive efficiency in livestock and improve assisted reproductive procedures in humans. It also has an application for the preservation of endangered species.

A patent application has been filed covering the use of retinoids in assisted reproductive procedures with the assistance of the UT Research Corporation. Dr. Godkin's research has received support from the Center of Excellence which has been leveraged into substantial funding from the USDA's National Research Initiative.

GASTRITIS PATHOGENESIS LABORATORY

Dr. Frank M. Andrews
Collaborators, staff and graduate students: Dr. A.G. Mathew, Dr. C.S. Patton, Dr. J.T. Blackford, Dr. A.M. Saxton, Dr. S. Murphy, Dr. J. Collins, Dr. R. Torres-Diaz, M. Sewell, A. Nadeau

Dr. Andrews studies the genesis and mechanisms of gastric ulcer development in a horse model. Gastric ulcer disease is a common problem in performance horses. The latter are commonly fed concentrated high-energy feeds that contain high levels of carbohydrates (starches). These carbohydrates undergo fermentation by resident bacteria in the stomach that may result in release of by-products such as volatile fatty acids (acetic, butyric, valeric and propionic acids). Previous research in this laboratory established that high concentrations of volatile fatty acids are produced in the stomach of horses fed high-energy diets. These acids, due to their high lipid solubility, diffuse into the non-glandular gastric cells causing acidification and damage to sodium transport, which leads to cellular injury and gastric ulceration.

Current studies involve examination of fresh viable non-glandular tissue (most susceptible to gastric ulceration) from the stomach of horses. Tissues are placed in an Ussing chamber system, which allows measurement of tissue short-circuit current (sodium flow) and resistance across the tissue. A decrease in short-circuit current and resistance are the first indicators of tissue damage, and precede gastric ulcer formation. These tissues then are viewed under the microscope after special staining to determine the nature and extent of cellular damage.

Dr. Andrews' research suggests that volatile fatty acids, especially butyric and propionic acids in contact with the stomach lining of horses and in the presence of stomach acid (pH = 1.5 and 4.0) leads to damage to cell's sodium transport system, cellular swelling, irreversible tissue damage and ulceration. These findings suggest that dietary modification, reducing the formation of these acids, may be helpful in prevention or decreasing the incidence of gastric ulcer disease in horses. These results have implications for several animal species including humans.

This research is funded by the Grayson-Jockey Club Research Foundation, the Comparative Gastroenterology Society and the Center of Excellence.

TALL FESCUE TOXICITY LABORATORY

Dr. Jack W. Oliver
Co-investigators and staff: Dr. R. Linnabary, Dr. E. Schultze and Dr. B. Rohrbach, L.K. Abney, E.M. Bailey, M. Cottrell and J. Czarra

Tall fescue toxicosis continues to be the primary grass-related disease in the United States in terms of economic loss to animal producers, affecting over 8.5 million beef cows and 700,000 horses. Tall fescue toxicosis is also a costly disease to Tennessee cattle producers, resulting in an approximate $100 million dollar annual loss due to unrealized production. Tall fescue is an attractive forage species because of its ability to withstand drought, poor soil conditions and intensive defoliation from grazing. It is grown on more than 34 million acres of pasture, but 75% of the pastures are infected with the endophytic-fungus, *Neotyphodium coenophialum,* at a sixty percent or greater level. Most of the infested pastures are in the Southeastern United States.

The endophyte-grass association results in the production of alkaloid toxins produced by the fungus or by the plant in response to the fungus. The alkaloids are biologically active causing a decrease in appetite and impaired reproduction and growth in animals. Endophyte-infected tall fescue has greater forage and seed productivity than the non-infected variety and is more drought tolerant. At the same time, tall fescue toxicosis is a costly disease to animal producers, causing severe reductions in weight gains, milk production and fertility.

Results of studies by Dr. Oliver's group have established that vascular damage is a central event that occurs when herbivores consume infected tall fescue. As a consequence of injury to blood vessels, blood flow to tissues is impaired causing localized tissue damage and thereby affecting the function of body systems. The abnormalities in blood flow are integrally related to the economic losses encountered by the cattle industry in the United States. Dr. Oliver has been examining toxicity associated with purified alkaloids that are suspected of being the primary tall fescue toxins (i.e. ergine, ergovaline). The long range goal of these studies is to understand how the individual toxic alkaloids cause damage to tissues of cattle because little is known regarding which of the alkaloids in tall fescue cause(s) the lesions in this syndrome.

Studies have been completed on analyses of various parameters of blood and tissues in steers that grazed endophyte-infected tall fescue over a three-year period. Markedly suppressed levels of serum copper were recorded in consecutive years, and copper deficiency may be the basis of the poor haircoats in these cattle. Gamma globulin (antibody) levels these cattle also were significantly reduced, suggesting that immunosuppression is an important aspect of the disease too. This information of tall fescue toxicity in cattle will be important in evaluating anti-fescue toxicosis treatments.

Continued research will be focused on the chronic effects of the two important

alkaloids of toxic tall fescue, ergine and ergovaline. Cattle will be treated with each of these alkaloids to evaluate their effect on the function of a specific blood vessel receptor (alpha-2 adrenergic). Dr. Oliver determined that ergovaline administration at the rate of 0.2 ug/kg/hour caused the typical decrease in the hormone (prolactin) in blood that occurs in cattle grazing on fungus-infected tall fescue pastures. Since the lining cells of blood vessels (endothelial cells) are damaged by exposure to the alkaloids, several inflammatory mediators associated with endothelial cell injury will be measured in the serum of the cattle that are infused with ergine or ergovaline.

Laboratory studies with isolated bovine endothelial cells or smooth muscle cells, grown in culture were treated with various concentrations of ergovaline and ergine. Both of these alkaloids are toxic to endothelial cells but ergovaline was considerably more potent. Dr. Oliver's results indicate that manipulation of the infection allowing ergine production in the plant but elimination of ergovaline presence would be beneficial. The ergine is necessary to convey insect resistance to tall fescue and the toxic effect would be minimized because the absence of ergovaline. Reducing the toxic effect of ergovaline in cattle will allow increased use of tall fescue, a forage with excellent nutrient quality, and root development that helps to control soil erosion. Dr. Oliver's research has been supported by the Center of Excellence, but his primary support is from the USDA's National Research Initiative.

BIOLOGICAL ACTIVITY TESTING AND MODELING LABORATORY

Dr. Terry W. Schultz
Staff and Graduate Students: G. Sinks, B. Gregory, J. Seward, and E. Hamblen

Research in the Biological Activity Testing and Modeling Laboratory focuses on the development of databases for structure-toxicity modeling and the development and validation of such models. The values of structure-toxicity models lie in their ability to predict toxic potency from molecular structure. This means that hazard assessment can be done while conserving time, manpower, resources, and animals.

Toxic potency is related to the uptake of the toxicant from the environment and its interaction with certain molecular sites of action. Since certain properties (such as hydrophobic, electronic, and steric factors) are related to molecular structure, Dr. Schultz's group focuses on identifying global descriptors of such properties that are best in modeling of uptake and interaction. Previous work by Dr. Schultz's group have shown that the site of action for toxic events is the cell membrane. In the case of covalent events, it is soft nucleophiles associated with membrane-bound proteins, whereas, in the case of non-covalent events it is the fatty acids of the membrane lipid bilayer.

This past year Dr. Schultz and his students have examined the ability of different molecular-orbital quantum-chemical parameters to quantify electrophilic reactivity. They have developed quantifiable chemical measurements for different types of interactions or bonding between the toxicant and its site of action.

Most recent work has focused on chemicals whose toxicity does not model well by this descriptor. Julie Seward, a senior doctoral student supported in part by COE funding, has shown that certain aromatic chemicals follow a unique model.

Other work in Biological Activity Testing and Modeling Laboratory has extended this approach to identify properties that best model toxic responses where interactions occur between the compound and a specific cell receptor. In the past year, this work focused on understanding the properties governing hormonal effects associated with the human estrogen receptor. Through an examination of the estrogenic activity of a series of selected chemicals using the *Saccharomyces crevice*-based *lac-Z* reporter assay, Dr. Schultz and his colleague were able to characterize and quantify the potency of estrogens that occur in the environment.

EXPERIMENTAL ONCOLOGY LABORATORY

Dr. Hildegard M. Schuller
Fellows, Staff and Graduate Students: Dr. H. K. Plummer III, Dr. Brian A. Jull, Dr. Y. Cakir, N. Neilsen, and K. Walker

Lung cancer is the leading cause of cancer deaths in all industrialized countries. East Tennessee has one of the highest lung cancer rates in the United States. Although cancers at other organ sites are more than twice as common, their cure rate is considerably higher. The most common cancer in men is prostate cancer, with a cure rate of 84%. Breast cancer is the leading type of cancer in women with a cure rate of 74%. In contrast, 158,700 (89.3%) of the 177,700 patients diagnosed with lung cancer in the year 1997 died within 12 months of diagnosis.

Smoking and exposure to second-hand smoke are the most intensively studied and best-documented risk factors for the development of lung cancer. Contrary to cancers at other organ sites, the incidence of lung cancer continues to rise in all industrialized nations. Moreover, teen smoking in the U.S. has increased at an alarming rate, thus setting the stage for even higher numbers of lung cancer cases 30-40 years from now. Another important contributing factor to the rise in lung cancer cases is the growing number of lung cancers developing in individuals never exposed to primary or second-hand smoke. This trend, which has been globally observed during the last two decades in all industrialized countries, is particularly evident for pulmonary adenocarcinoma. Of the six types of lung cancer recognized by the World Health Organization classification, two (small-cell carcinoma and adenocarcinoma) accounts for 90% of all lung cancers, but 30% of these cases do not have a history of exposure to primary or second hand smoke.

The lung cancer "epidemic" is closely related to a globally observed rise in chronic lung diseases such as bronchitis, bronchiolitis, asthma, emphysema, and chronic obstructive pulmonary disease. This disease complex, which is often referred to as "allergies" has the same geographic distribution as lung cancer with which it shares some risk factors such as smoking and air pollution. Accordingly, East Tennessee, which has

one of the highest lung cancer rates in the U.S., is also often referred to as "the land of allergies." For all lung cancer types, chronic lung disease has been identified as a risk factor even without a history of exposure to smoke.

Dr. Schuller's research has been dedicated to the study of lung cancer for over 20 years. It is her belief that effective strategies for the prevention and therapy of this disease complex can only be based on an in-depth understanding of the regulatory mechanisms which govern the growth of normal lung cells and the cancers arising from such cells. In contrast to other laboratories that are searching for the "magic molecular event" responsible for the genesis of all lung cancers, she hypothesized that different lung cell types and different types of lung cancer may be governed by different regulatory mechanisms, which in turn may be affected differently by known risk factors for the disease.

Dr. Schuller's achievements in lung cancer research have been recognized nationally and internationally. Her research has been supported by the Center of Excellence, but her primary support comes from substantial grants of the National Cancer Institute and the pharmaceutical industry.

Dr. Schuller's group previously determined that the growth of small cell lung carcinoma and the cell of origin of this cancer type (pulmonary neuroendocrine cell) is regulated by a specific cell surface receptor (nicotinic acetylcholine) which has an important biochemical function (calcium channel). She found also that the tobacco-specific carcinogenic product (NNK) activates this receptor with high affinity. Binding of this product to the receptor causes a release of a biochemically active substance (serotonin) by these cells and that this substance markedly stimulates cell division when it is taken up by other cells. This is an important finding because it links, for the first time, the stimulation of a specific receptor by a tobacco-specific toxicant resulting in the activation of a series of cell-specific events that may result in uncontrolled growth. Experiments are now underway to test the hypothesis that substances which inhibit the re-uptake of serotonin will protect against the development and spread of small cell lung carcinoma. These drugs are already approved for the treatment of psychiatric diseases and migraine and could immediately enter clinical trials in smokers and small cell carcinoma patients.

Laboratory studies with various cultures of small cell lung cancer cells and pulmonary neuroendocrine cells suggest that smoking or chronic exposure to the product NNK, increases the concentration of the target receptors on these cells. Dr. Schuller now is working with Dr. Kabalka of the Department of Chemistry on the development of novel cancer imaging agents which selectively bind with high affinity to this receptor that will allow for a selective and highly sensitive detection of small cell carcinomas in people (by positron emission tomography). This will constitute an important clinical application of her research. The Center's emphasis on Molecular Genetics and Carcinogenesis and Dr. Schuller's work and collaboration with various investigators has culminated in a Program Grant Proposal to NIH for $12,000,000.00 (see section on "Research Funding").

ANTICANCER MOLECULAR ONCOLOGY LABORATORY

Dr. Hwa-Chain R. Wang
Fellows: K. Fecteau, J. Mei, Y. Sun, M. Tan

Dr. Wang's long-term research goals concerns tumor-specific intracellular molecular signaling networks and to uncover signaling pathways that can be induced by anticancer agents to lead cancerous cells into programmed cell death (apoptosis).

Short-term goals are to identify intracellular signaling elements whose activation is involved in induction of apoptosis or growth inhibition of cancer cells. A corollary to this is to identify novel anticancer agents, which may selectively induce apoptosis of cancer cells while sparing normal cells. Ultimately, he expects to apply the understanding of intracellular signaling control to anticancer therapeutics and prevention.

Currently, Dr. Wang focuses on three projects. The first is to understand the molecular and cellular function of a family of novel intracellular enzymes (Krs and QIK), which are induced in resting cells and cells undergoing programmed cell death. The second is to study molecular and biological activities of a novel natural anticancer agent (FR901228), which selectively induces programmed cell death of cancer cells. The third is to study the molecular and biological roles of mutations in genes that control tumors (suppressor genes - transforming growth factor beta receptor genes, *TβR*-II and *TβR*-I) in the development of human oral cancers.

Dr. Wang has identified a family of novel enzymes (kinases: SAMK/Krs1 and QIK) that are activated in normal resting cells and in cancerous cells undergoing programmed cell death (as a result of a variety of physiological, chemical or physical stresses). Induction of QIK activity is involved in establishing cell quiescence but additional activation may result in cell death. He is investigating the molecular and biological roles of these enzymes in cancer development and programmed cell death. Uncovering the apparent novel signaling pathway that cross-links cancer development of cells to programmed cell death should be directly exploitable for development of anticancer therapeutics.

Investigation into the molecular mechanisms of potential anticancer therapeutic agents on a variety of cancer cell types particularly human breast cancer cells is ongoing. Cancerous mouse embryo cell cultures and various human tumor cell cultures are used to screen anticancer agents. Studies on the molecular effects of a novel natural anticancer agent (FR901228) on different intracellular metabolic signaling pathways should uncover the mechanism of this agent to selectively induce programmed cell death in cancerous cells. Dr. Wang determined that at least five important intracellular metabolic signaling pathways including the QIK pathway are affected. The information will be a basis for clinical trials using FR901228 in combination with other anticancer agents.

The role of mutations in tumor suppressor genes (*TβR*-II and *TβR*-I genes) in the multi-stage development of human oral cancers will require the detection and analysis of

the mutations in these genes isolated from pre-malignant and malignant cells of the oral cavity. Specific mutations in the *TβR*-II and *TβR*-I genes will be identified by their functional inability to transmit signals to suppress tumor formation. This research is a part of a major collaborative program project to study molecular events in progression and prevention of oral cancer.

Dr. Wang's research is supported by the Center of Excellence, but his primary funding source is the National Institutes of Health (National Cancer Institute and National Institute of Dental Research).

CANCER MODELLING LABORATORY

Dr. Michael McEntee
Collaborators, Staff and Graduate Students: Dr. J. Whelan, A. Cruikshank, N. Neilsen

Dr. McEntee's research focuses on defining the relationship between tissue levels of polyunsaturated fatty acids, their metabolism as bioactive lipids (such as prostaglandin E_2), and forms of cancer to which they have been linked. In collaboration with a biochemist in the Department of Nutrition at UT, Dr. J. Whelan, he demonstrated recently that specific dietary polyunsaturated fatty acids can significantly protect against the development of intestinal cancer in a mouse model of the human disease. Non-steroidal anti-inflammatory drugs like aspirin inhibit the metabolism of polyunsaturated fatty acids into various prostaglandins. Their research involved the simultaneous pharmacologic and dietary manipulation of tissue polyunsaturated fatty acids concentrations.

Polyunsaturated fatty acids derived from fish oils reduced the incidence of this form of neoplasia by 50% in comparison to polyunsaturated fatty acids common in the U.S. diet (i.e. animal fat and vegetable oil). Their research suggested that this protective effect was specifically attributed to longer chain, highly unsaturated polyunsaturated fatty acids. Dr. McEntee also demonstrated for the first time in an animal that prostaglandins produced from the "bad" tissue polyunsaturated fatty acids specifically contributes to intestinal tumor growth. Inhibition of the metabolism of polyunsaturated fatty acids found in corn oil and red meat to prostaglandins significantly contributes to intestinal carcinogenesis.

The importance of prostaglandins in production of tumors was subsequently confirmed in experiments where tumors were eliminated following treatment with an antibody that specifically inactivates this polyunsaturated fatty acid product. Prostaglandin E_2 acts through specific cell receptors and they have shown that it is produced by the non-neoplastic part of intestinal tumors of their mouse model (as it is in humans). They are currently attempting to characterize the distribution of the prostaglandin E_2 receptors in these lesions in order to better understand the link between the production/target activity of this specific bioactive lipid and intestinal carcinogenesis. Recent data suggest that the molecular changes contributing to intestinal carcinogenesis

in humans and their mouse model also occur in pet dogs that develop this form of neoplasia. This strongly implies that the beneficial effects of dietary and pharmacologic intervention demonstrated in the mouse model would directly translate to dogs, as well as humans.

In addition to the above experiments, studies have been initiated to investigate the contribution of polyunsaturated fatty acids and their metabolites to another common form of neoplasia that has been strongly linked to dietary fats in humans, prostatic cancer.

Dr. McEntee receives support from the Center of Excellence, but his primary funding is derived from the Department of Defense, American Institute of Cancer Research and Monsanto.

CELL BIOLOGY LABORATORY

Dr. Xuemin Xu
Staff and Graduate students: G. Mao, Ph.D., Y. Shi, W. Gao, and E. Laag

The long-term goal of Dr. Xu's research is to understand the molecular and cellular mechanism of Alzheimer's disease, and the formation of senile plaques, the pathological hallmark of this disease. His group is conducting two projects. One is to determine the pathological function of a substance (presenilin 1) in brain degeneration and the genesis of another substance (amyloid) observed in Alzheimer's disease. The other project is to determine the role of a certain protein (apolipoprotein E) in the formation and clearance of another protein involved Alzheimer's disease (β-amyloid peptide).

Alzheimer's disease is a progressive degenerative disorder, characterized by memory loss, confusion, and a variety of cognitive disabilities. An estimated four million American suffer from Alzheimer's disease. It is the fourth major cause of death in the United States following heart disease, cancer, and stroke. Alzheimer's disease is the third most costly disease in the U.S. With the rapid growth of the senior population, Alzheimer's disease poses, besides its tragic personal impact, serious problems to families, caregivers, government and health care in institutions.

Molecular genetic analysis of familial (heritable) Alzheimer's disease has led to the identification of three Alzheimer's disease-causative genes, those of β-amyloid precursor protein, presenilin 1, and presenilin-2. A fourth gene encoding apolipoprotein E has also been associated with Alzheimer's disease as a risk factor but not as a causative gene for Alzheimer's disease. Among these Alzheimer's disease -causative genes, mutations in presenilin 1 gene account for the majority of the known cases of familial Alzheimer's disease. Presenilin 1 has been implicated in two pathological events: (1) the generation of amyloid-β peptide, which is the building block of the toxic "plaques" characteristic of brain tissue from patients with Alzheimer's disease, and (2) programmed cell death, or apoptosis, a natural process in which unneeded or worn-out cells commit suicide. However, questions regarding the mechanisms by which the mutations in presenilin 1 proteins alter β-amyloid precursor protein processing and cause programmed cell death, as well as the normal function of presenilin 1, remain to be answered, which is the goal of their current research project.

Recent work resulted in the identification of a novel molecule (PSAP) which is capable of inducing programmed cell death, one of the mechanisms of neuronal cell death observed in Alzheimer's disease brains. It reacts specifically with presenelin 1. This finding established for the first time the molecular link between presenelin 1 and programmed cell death. Currently, we are conducting the experiments to determine the role presenelin 1 has in regulating PSAP-induced programmed cell death and with other Alzheimer's disease protein structures such as amyloid plaques and neurofibrillary tangles. This study will contribute to understanding the pathological function of presenilin 1 and will provide new insight into the mechanism of Alzheimer's disease. This study may also lead to the identification a new therapeutic target of Alzheimer's disease treatments.

Dr. Xu's studies in exploring the role of apolipoprotein E in β amyloid peptide formation suggests that these substances bind together to form complexes that interfere with the function of a critical enzyme α-secretase. The binding of apolipoprotein E to newly generated β amyloid peptide also may play a role in determining whether the latter is deposited or cleared. These studies will lead to a better understanding of the mechanism by which the apolipoprotein E is involved in Alzheimer's disease.

Dr. Xu's experiments are conducted with laboratory models, including brain (glial) cells containing the genes of apolipoprotein E and for amyloid precursur protein and in a yeast two-hybrid system. Because mutations in the presenilin 1 gene are associated with the majority of familial Alzheimer's disease, these studies may provide important information for the early diagnosis and therapy of this disease.

Dr. Xu's work is funded by grants from the National Institutes of Health, Sigma Kappa and by the Alzheimer's Association. He receives support also from the Center of Excellence.

VASCULAR BIOLOGY LABORATORY

Dr. Mei-Zhen Cui
Staff: Guojun Zhao

Dr. Cui's work involves the understanding of mechanisms underlying the development of vascular diseases including atherosclerosis and thrombus development. Specifically, their project is focused on elucidating the mechanism by which certain substances (lipoprotein and lipid) regulates production of an important protein (tissue factor) involved in blood clotting. Knowledge of how lipoproteins regulate tissue factor could provide new insights into heart attacks since blood clots and high levels of lipoproteins worsen the disease leading to the heart attacks.

Tissue factor is a cell surface protein that initiates blood clotting and regulates thrombosis. Enhanced tissue factor production has been observed in smooth muscle cells in atherosclerotic lesions of blood vessels and in balloon-damaged arteries. It may accelerate a variety of pathological processes in vessel walls. A high level of a certain

protein in blood [plasma low density lipoprotein (LDL)] is a risk factor for developing atherosclerosis. However, the relationship among increased lipoprotein, tissue factor expression and atherosclerosis is not well understood.

They have found that tissue factor production is enhanced by native and oxidized LDL. This finding suggests that one of the mechanisms by which lipoprotein causes atherosclerosis is to induce tissue factor production. The objective of their studies is to determine how lipoproteins, which are large molecules that carry cholesterol and fats in blood, cause tissue factor to be produced. The hypothesis is that the way lipoproteins induce tissue factor in cells is distinct from other substances that induce it. They are trying to define that novel pathway. Most of their discoveries have been made in a cell that is typically involved in vascular disease, the smooth muscle cell. Various molecular and genetic approaches have been used to demonstrate that lipoproteins significantly induced tissue factor production. Their findings have important implications for a connection between lipoproteins and tissue factor in development of atherosclerotic lesions. They hope to elucidate factors promoting the development of vascular diseases and that may suggest novel therapeutic approaches. Recently the Center of Excellence has started supporting Dr. Cui's research that is primarily funded by the American Heart Association.

DISSEMINATION OF RESEARCH TO THE GENERAL PUBLIC

Dr. Nancy Howell

One important function of the Center of Excellence is to provide information to the general public. This information may increase the public's awareness of research and may provide individuals with valuable results that may improve their lives or their agribusiness.

To distribute information the College of Veterinary Medicine uses several methods. A general newsletter is distributed twice a year throughout Tennessee and beyond, highlighting research activities. Features concerning on-going research, in addition to results from concluded research projects, are included in the publication, *Veterinary News*, which is written for general audiences. Features also appear in other University of Tennessee publications, including *UT Agriculture*, *UT Alumnus* and *Tennessee AgriScience*.

In addition, news releases are distributed to state media and to regional and national media. Television and print publications produce numerous features about the College each year, many related directly to research conducted through the Center of Excellence. Public displays about the College frequently include highlights of COE research. Center of Excellence researchers are invited to share their research not only professionally, but as speakers to commodity groups, civic groups and other interested individuals.

Research is a component of the College's web site, including COE projects such as the tall fescue toxicity research and other research projects.

Dissemination of research results through the news media helps inform the public and provides citizens with a better understanding of the practical applications of science in their daily lives.

PLANS FOR THE FUTURE

Strategic Planning and External Review.

The support provided by the Center for some of our promising investigators undoubtedly will constitute the embryogenesis of established research programs. It is anticipated that the extramural funding and other benchmarks will make an incremental jump again this year as a result of our strategic recruiting over the past two years (see "Funding Levels").

Dr. G. Michael Shires, Dean and Director of the Center of Excellence in Livestock Diseases and Human Health retired at the end of the 1999-2000 fiscal year. The new Dean of the College of Veterinary Medicine, Dr. Michael Blackwell, will serve as the new Director of the Center. Because the Dean of the College of Veterinary Medicine has announced the intention to add an Associate Dean for Research to his administration team, the Assistant Director of the Center likely also will be replaced in 2001. This will represent new management of the Center.

The change in leadership should constitute an opportune time for reviewing the program. Since the Center for Livestock Diseases and Human Health was determined to be an established Center in 1988, an external review has been done every four years. The last review was in 1996 and with the departure of the Center's Director, Dean G. Michael Shires, in 2000 and the assumption of new leadership by the new Dean of the College, Dr. Michael Blackwell, an external review is being considered for 2001. Preliminary plans are to invite a team of four experienced researchers from Colleges of Veterinary Medicine and appropriate research institutions for review of the program and to make recommendations. The latter will be used to assist in strategic planning by the Center's leadership with assistance of core faculty.

Some issues that ought to be considered in a review of the Center include whether the focus areas of the Center are still appropriate and addressing the needs of society and our stakeholders in Tennessee. Another challenge is for the Center to address the replacing of critical aging equipment and upgrading equipment for molecular research. These include ultracentrifuges, high performance liquid chromatography equipment, gas chromatography unit , quantitative PCR and atomic spectrometer. The flexibility of utilizing the Center's funds to interact with other funded Centers at the University of Tennessee ought to be considered. This includes the need for a transgenic mouse facility.

Coping with a 10% Reduction in Funding:

A 10% reduction in funding will substantially impinge on the research productivity of the center. The funding levels over the last five years have increased very modestly (8.9% for an annual average of 1.8%) but have not kept abreast with inflation. Fortunately our faculty have been productive and funding levels have significantly increased especially in the last part of this reporting period. A reduction in funding must be addressed with an appropriate strategic plan. A reduction in funding can be addressed perhaps by only two mechanisms, a 10% reduction in all categories of expenditures (or deeper cuts in specific areas such as elimination of equipment support) or by elimination of one of the focus areas of the Center. The first option is not advisable because many projects are only partially funded which jeopardizes the potential for successful leverage into extramural support by the investigator. For the second option, it is envisaged that the priority of the five focus areas of focus will be revisited. The focus area(s) with the lowest priority then could be dropped with respect to Center support so that the Center can continue to focus on, support and promote the remaining areas. This will be a difficult decision because investigators in each of its focus areas continue to generate extramural funding.

Coping with a 10% Increase in Funding:

The Center functions to enhance research in the targeted areas and to leverage Center funds to help recruit strategic hires. This is achieved by assisting with start-up packages for new faculty and by providing seed money for existing faculty. Additional funding could be used to fund an additional focus area or to fund existing areas more completely than before. The former would require review of existing focus areas and of the potential of adding new areas such as emerging zoonotic diseases and/or food safety. However, the second option may be more appealing. Annually, appropriate proposals are solicited and evaluated using criteria to support the objectives of the Center. Many excellent proposals are received and several are rejected. Most are not fully funded which impairs the ability of investigators to leverage Center funds into extramural support. A strategic plan, including the recommendations of an external review would be the first step in considering the wisest application of increased funding. However, it is evident that fully funding some or most of the proposals with a high review score may be the most productive investment of increased funding for the Center.

TABLE 1

CENTER OF EXCELLENCE IN LIVESTOCK DISEASES AND HUMAN HEALTH BENCHMARKS OF FACULTY ACCOMPLISHMENTS

1995-2000

	Year 12 (Year 07 as Accomplished Center) 1995-1996		Year 13 (Year 08 as Accomplished Center) 1996-1997		Year 14 (Year 09 as Accomplished Center) 1997-1998		Year 15 (Year 10 as Accomplished Center) 1998-1999		Year 16 (Year 11 as Accomplished Center) 1999-2000	
	Actual	Avg	Actual	Avg	Actual	Avg	Actual	Avg	Actual	Avg
Number of:										
Articles	153	(7.29)	176	(7.33)	179	(11.2)	131	(9.4)	89	(5.24)
Books or Book Chapters	5	(0.27)	5	(0.21)	104	(6.5)	16	(1.1)	10	(0.63)
P ublished Proceedings	65	(2.95)	71	(2.96)	72	(4.5)	42	(4.5)	18	(1.13)
Total Publications	223	(10.62)	249	(10.38)	355	(22.2)	189	(22.2)	117	(7.31)
Abstracts	64	(3.05)	71	(3.01)	56	(3.5)	71	(3.5)	20	(1.25)
Invited Participation at:										
Regional Meetings	55	(2.62)	68	(2.83)	76	(4.8)	43	(4.8)	53	(3.31)
National Meetings	70	(3.18)	76	(2.92)	74	(4.6)	62	(4.6)	44	(2.75)
Faculty in Center	22		24		16		14		16	
Number of Visitors	18		18		19		11		12	

TABLE 2

RESEARCH PROJECTS FUNDED EXTERNALLY

REPORT PERIOD 1999-2000

PROJECT DIRECTOR	TITLE OF GRANT	FUNDING AGENCY	TOTAL AWARDED	ESTIMATED EXPENDITURES
Frank Andrews	Pathogenesis of acid injury in the glandular and non-glandular region of the equine stomach.	Comparative Gastroenterology Society Research	$5,000.00 07/01/98-07/01/00	0
	Pathogenesis of acid injury in the non-glandular region of the equine stomach.	Grayson-Jockey Club Research Foundation	$37,110.00 04/01/99-03/31/01	$24,035.58
Joseph Bartges	Funding for a summer veterinary student and a clinical project: Determination of maintenance energy requirements of client-owned dogs	Ralston Purina	$10,000.00 05/24/99-08/31/00	$2,091.86
	Comparison of two dietary approaches for managing canine chronic renal failure	Iams	$29,522.00 04/01/99-07/01/02	0
	Pentosan polysulfate sodium in the treatment of idiopathic feline lower urinary tract disease.	DVM Pharmaceuticals	$133,112.00 09/12/97-08/31/01	$58,909.35
	Influence of diet on urine saturation with struvite in healthy cats	Ralston Purina	$9,594.00 10/01/97-10/31/00	$4,824.59
	Influence of diet on glycemic control in dogs with spontaneous insulin-dependent diabetes mellitus	Ralston Purina	$6,093.00 10/01/97-10/31/00	$4,463.88
	Influence of alkalinization on urinary saturation with calcium oxalate and struvite, and bone mineral density in cats	Morris Animal Foundation	$25,000.00 09/01/97-03/31/00	$8,079.07
	APR studies	Hills Pet Nutrition	$13,500.00 11/01/97-10/31/99	0
David Bemis	Antibody response to *Bordetella bronchiseptica* chimeric fimbrial protein antigen	USDA 1433 Funds	$12,000.00 10/01/98 – 09/30/99	$2,354.31

David Brian	Bovine coronavirus vector for mucosal immunity to phaemolytica leukotoxin	USDA	$140,000.00 09/15/95-09/30/00	$55,444.58
	Mechanism(s) of coronavirus RNA replication and packaging	National Institute of Allergies and Infectious Diseases	$586,309.00 07/01/96-06/30/01	$70,420.63
Mei-Zhen Cui	Scientist development award	American Heart Association	$240,000.00 01/01/98-12/31/01	$48,889.97
James Godkin	The role of retinol in oocyte maturation and early embryonic development.	USDA	$215,000.00 09/01/99-08/31/03	$19,969.00
Stephen Kania	Serum neutralization of bovine virus	Pfizer	$6,400.00	$6,887.54
	Laboratory tests for vaccine development	Pfizer	$5,000.00 7/15/99-7/31/00	$13,701.21
	Ophidian paramyxovirus infection	American Zoo & Aquarium Assoc	$16,900.00 10/01/98-9/30/00	$11,739.49
	A recombinant protein based TSH assay	American Kennel Club	$33,796.00 05/01/97-09/30/99	$28,220.00
Alan Mathew	Effects of environment and management on persistence of antibiotic resistance in bacteria from swine	The International Life Science Institute	$62,789.00 09/01/99-08/31/00	$18,771.00
	Resistance patterns on swine farms using or excluding antimicrobial products	National Pork Producers Council	$22,490.00 10/01/98-12/31/99	$15,549.00
	Effect of drug combinations and regimens of antibiotic resistance	National Pork Producers Council	$24,090.00 10/01/98-09/30/99	$1,812.00

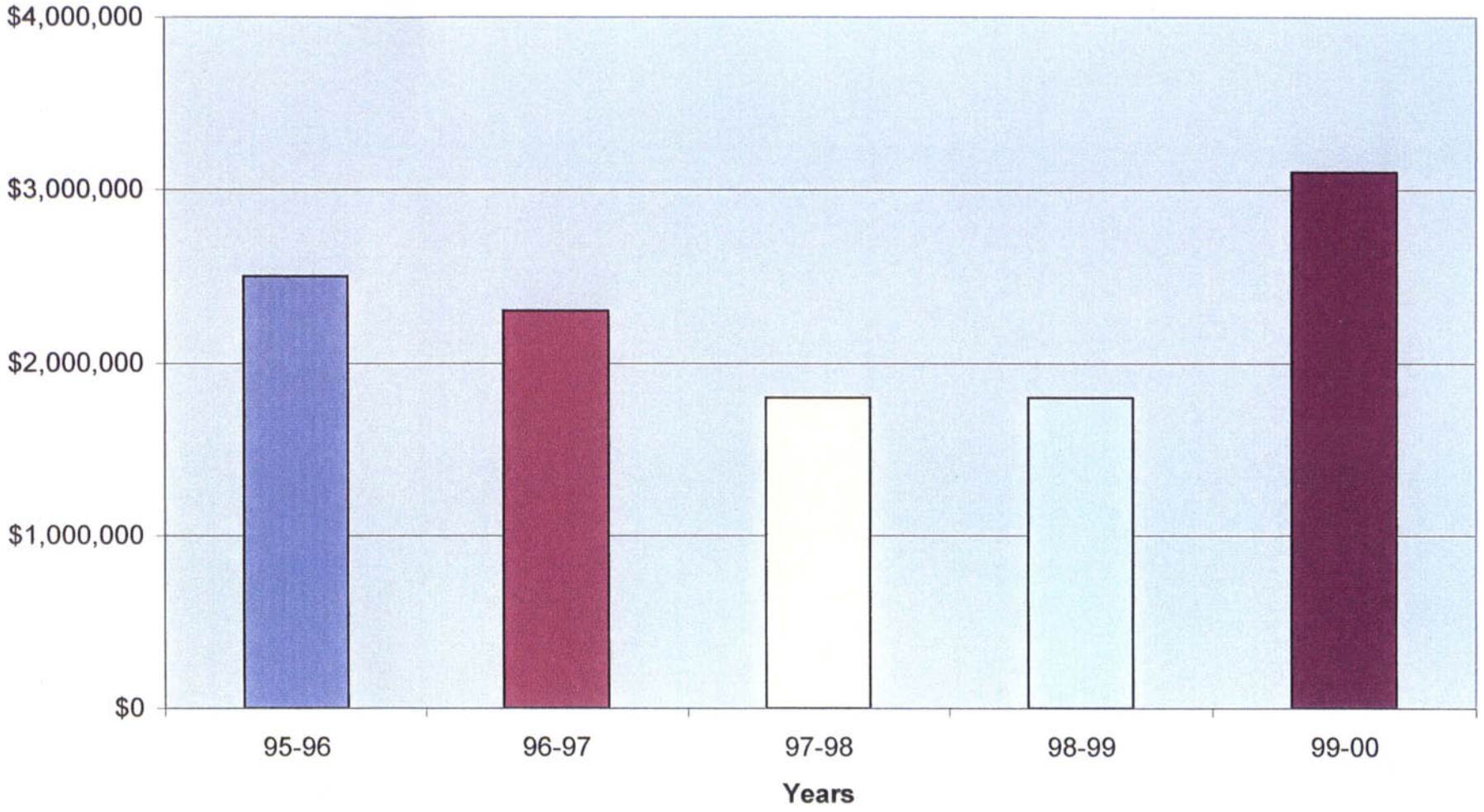
Michael McEntee	Effect of n-3 and n-6 polyunsaturated fatty acids on growth and progression of prostatic cancer in vivo	DOD-Army	$312,938.00 04/15/00-05/14/03	$940.36
	In vivo effects of resveratrol during early stages of intestinal tumorigenesis	American Institute of Cancer Research	$75,952.00 07/01/99-06/30/01	$12,302.51
	Dietary effects of a variety of fatty acid ethyl esters on the formation and development of intestinal polyps in the Min/+ mouse model when provided in the context of a western diet	Monsanto (Co-PI)	$71,656.00 01/01/99-01/01/00	$71,656.00
	Delta-6 desaturase inhibition and intestinal tumorigenesis	Monsanto (Co-PI)	$7,150.00 6/99-1/00	$7,150.00
	Role of arachidonic acid and PGE2 as key mediators of intestinal tumorigenesis in vivo	American Institute of Cancer Research (Co-PI)	$158,484.00 7/99-6/01	$82,000.00
John New	The feasibility of behavioral counseling to promote successful puppy adoptions	Morris Animal Foundation	$34,700.00 07/16/97-06/30/99	$7,350.39
	The feasibility of behavioral counseling to promote successful puppy adoptions	PetsMart Charities	$10,000.00 10/01/97-09/30/99	0
Jack Oliver	Reactivity of bovine vasculature to Ergovaline and Ergine of toxic tall fescue	USDA	$188,000.00 10/01/97-09/30/00	$87,731.35
	Assessment of inflammatory and immunologic response of cattle to tall fescue toxins	USDA 1433 Funds	$3,000.00 10/01/98-9/30/99	$500.00
	Pathophysiologic studies of tall fescue toxins in cattle	USDA 1433 Funds	$6,000.00 10/1/99-09/30/00	$389.31

Stephen Oliver	Rapid, specific test for salmonella subtypes	National Pork Producer's Council	$29,150.00 06/01/99-07/31/00	$26,221.00
	Safety specific immune responses and protection in experimentally infected dairy cows	Pfizer	$165,000.00 04/01/99-12/31/00	$1,400.00
	Reproductive performance and reproductive disorders in Jersey cows	American Jersey Cattle Club	$6,250.00 04/01/99-04/01/00	$46,812.00
	Development of chronic streptococcus uberis intramammary infections in lactating dairy cows	Pharmacia and Upjohn	$56,258.00 01/01/99-12/31/00	$15,341.00
	Evaluation of specific immune responses and protection by streptococcal uberis antigens in naturally infected dairy cows	Pfizer	$245,624.00 06/01/98-12/31/99	$64,318.00
	Evaluation of the Uterine Environment During Clinical Mastitis in Lactating Jersey Cows	American Jersey Cattle Club	$5,500.00 04/01/00-04/01/01	$2,010.00
	Model for Determining Effects of Mastitis on Reproductive Performance in Jersey Cattle	American Jersey Cattle Club	$4,250.00 04/01/99-03/31/00	$4,250.00
	Evaluation and use of BAM/FDA and rapid microbiological methods for on-farm surveys	Food and Drug Administration	$475,610.00 09/30/98-09/29/01	$120,545.71
Sharon Patton	Field Test for Diagnosing Toxoplasma gondii in Swine	National Pork Producer's Council	$18,435.00 10/01/98-06/30/00	$13,265.22

Barry Rouse	Herpes zosterfilization	Smith-Kline Biological	$124,746.00 open	$8,572.72
	Immunity mechanisms in herpesvirus infections	National Institute of Allergy and Infectious Diseases – NIH	$1,065,526.00 06/01/95-05/31/00	$225,460.14
	Mechanisms of herpetic stromal keratitis	National Eye Institute – NIH	$1,296,345.00 09/30/97-09/29/02	$205,778.43
	Biodelivery sciences	Biodelivery Sciences	$12,000.00 open	$3,252.27
	HSP peptide complexes as a putative vaccine against herpes simplex virus	Antigenics Agency	$16,620.00 04/15/99-04/14/00	0
	Vaccination against herpes simplex virus	National Institute of Allergy and Infectious Diseases- NIH	$1,396,346.00 03/01/00-02/28/05	0
Hildegard Schuller	Transplacental pancreatic carcinogenesis by NNK	National Institute of Health	$1,001,479.00 08/01/96-07/31/01	726,399.83
	Effects of the phosphodiesterase inhibitor B9302-107 on the nasal cavity in hamsters, mice and rats	BYK Gulden	$40,360.00 04/01/98-03/31/00	$40,360.00
	FACS Vantage SE Cell Sorter/Flow Cytometer	National Center for Research Resources	$150,000.00 04/01/00-03/31/01	0

Terry Schultz	Development of a bioremediation risk assessment scheme	US Environmental Protection Agency	$59,199.00 09/94-09/98	$20,000.00
	Microbial transformation and molecular toxicology of estrogens	Water Resources Research Institute	$295,334.00 12/07/98-12/06/00	$64,036.00
	The Role of bioavailability in determining acceptable limits for the bioremediation of polychlorinated biphenyls	U.S. Department of Energy	$441,037.00 01/01/97-05/31/00	$160,053.00
	Ecotoxicity of organic chemicals	Proctor and Gamble	$6,600.00 11/0199-10/31/01	0
	Degradation of natural estrogens in wastewater treatment facilities	University of Mississippi	$25,400.00 09/01/97-10/31/00	$25,397.00
David Slauson	Cellular Pathobiology of Environmental Disease	NIH	$590,897.00 07/01/95–06/30/00	$218,931.17
Carla Sommardahl	Molecular analysis of PKD in the TGN737RPW mouse	NIH	$422,121.00 07/01/97-07/01/01	$177,474.72
Hwa-Chain Wang	Pathway leads to apoptosis in SCR-transformed cells	NIH	$517,520.00 01/01/98–12/31/02	$128,162.21
	Biochemical evaluation of the functionality of mutated TBR-II and TBR-I receptors	Ohio State University	$192,453.00 09/15/98-06/30/03	$26,755.67
Xuemin Xu	Role of APOE in beta amyloid formation	Sigma Kappa	$30,000.00 11/01/98-10/31/00	$10,245.86
	Role of apoE App processing	Alzheimer's Association	$80,000.00 08/01/98-12/31/00	$2,425.21
	Role of apolipoprotein in AD amyloid formation	National Institute of Neurological Disease and Stroke	$677,968.00 05/01/99-04/30/02	$99,354.58
TOTALS			**$11,946,613.00**	**$3,103,004.72**

Figure 1
CENTER OF EXCELLENCE FOR LIVESTOCK DISEASES AND HUMAN HEALTH EXTERNAL FUNDING EXPENDITURE LEVELS FOR 1995-2000



CENTERS OF EXCELLENCE/CENTERS OF EMPHASIS
ACTUAL, PROPOSED, AND REQUESTED BUDGET

Institution: Veterinary Medicine Center: Livestock Diseases and Human Health

	1995-96 Actual			1996-97 Proposed			1997-98 Requested		
Expenditures	252,995	506,403	759,398	266,898	533,797	800,695	274,575	549,150	823,725
Salaries									
Faculty	31,287	62,668	93,955	29,637	59,363	89,000	30,969	62,031	93,000
Other Professional	50,555	101,262	151,817	35,118	70,343	105,461	37,296	74,704	112,000
Clerical/ Supporting	20,937	41,939	62,876	20,488	41,039	61,527	21,645	43,355	65,000
Assistantships	10,027	20,081	30,108	19,927	39,915	59,842	20,446	40,954	61,400
Total Salaries	112,806	225,950	338,756	105,170	210,660	315,830	110,356	221,044	331,400
Fringe Benefits	25,763	51,603	77,366	27,992	52,672	80,664	27,306	54,694	82,000
Total Personnel	138,569	277,553	416,122	133,162	263,332	396,494	137,662	275,738	413,400
Non-Personnel									
Travel	1,029	2,062	3,091	1,332	2,668	4,000	1,332	2,668	4,000
Software	2,234	4,475	6,709			0			0
Books & Journals	450	904	1,354			0			0
Other Supplies	44,653	89,088	133,741	73,488	147,197	220,685	69,430	138,245	207,675
Equipment	60,834	121,852	182,686	51,615	103,385	155,000	56,610	113,390	170,000
Maintenance	4,772	9,560	14,332	6,901	16,415	23,316	8,741	17,509	26,250
Scholarships	454	909	1,363	400	800	1,200	800	1,600	2,400
Consultants			0			0			0
Renovation			0			0			0
Other (Specify)			0			0			0
			0			0			0
			0			0			0
			0			0			0
Total Non-Personnel	114,426	228,850	343,276	133,736	270,465	404,201	136,913	273,412	410,325
GRAND TOTAL	252,995	506,403	759,398	266,898	533,797	800,695	274,575	549,150	823,725
Revenue									
New State Appropriation		517,200	517,200		523,000	523,000		549,150	549,150
Carryover State Appropriation			0		10,797	10,797			0
New Matching Funds	258,600		258,600	261,500		261,500	274,575		274,575
Carryover from Previous Matching Funds			0	5,398		5,398			0
Total Revenue	258,600	517,200	775,800	266,898	533,797	800,695	274,575	549,150	823,725

CENTERS OF EXCELLENCE/CENTERS OF EMPHASIS
ACTUAL, PROPOSED, AND REQUESTED BUDGET

Institution llege of Veterinary Medicine Center Livestock Diseases and Human Health

	1996-97 Actual			1997-98 Proposed			1998-99 Requested		
	Matching	Appropr.	Total	Matching	Appropr.	Total	Matching	Appropr.	Total
Expenditures	257,150	514,300	771,450	251,550	503,100	754,650	264,127	528,255	792,382
Salaries									
Faculty	29,542	57,084	86,626	28,333	56,667	85,000	29,750	59,500	89,250
Other Professional	35,326	72,653	107,979	35,888	71,775	107,663	37,682	75,364	113,046
Clerical/ Supporting	22,043	44,087	66,130	9,927	19,855	29,782	10,423	20,848	31,271
Assistantships	13,482	26,963	40,445	15,413	30,826	46,239	16,184	32,367	48,551
Total Salaries	100,393	200,787	301,180	89,561	179,123	268,684	94,039	188,079	282,118
Fringe Benefits	22,122	44,243	66,365	21,095	42,190	63,285	22,150	44,299	66,449
Total Personnel	122,515	245,030	367,545	110,656	221,313	331,969	116,189	232,378	348,567
Non-Personnel									
Travel	518	1,035	1,553	1,278	2,557	3,835	1,342	2,685	4,027
Software	279	559	838			0			0
Books & Journals	99	197	296			0			0
Other Supplies	42,955	85,910	128,865	73,654	147,309	220,963	77,337	154,674	232,011
Equipment	82,467	164,934	247,401	50,843	101,685	152,528	53,385	106,769	160,154
Maintenance	4,267	8,535	12,802	7,452	14,903	22,355	7,824	15,649	23,473
Scholarships	7,278	14,555	21,833	7,667	15,333	23,000	8,050	16,100	24,150
Consultants			0			0			0
Renovation			0			0			0
Other (Specify)			0			0			0
			0			0			0
			0			0			0
			0			0			0
Total Non-Personnel	137,863	275,725	413,588	140,894	281,787	422,681	147,938	295,877	443,815
GRAND TOTAL	260,378	520,755	781,133	251,550	503,100	754,650	264,127	528,255	792,382
Revenue									
New State Appropriation		514,300	514,300		503,100	503,100		528,255	528,255
Carryover State Appropriation		6,455	6,455			0			0
New Matching Funds	257,150		257,150	251,550		251,550	264,127		264,127
Carryover from Previous Matching Funds	3,228		3,228			0			0
Total Revenue	260,378	520,755	781,133	251,550	503,100	754,650	264,127	528,255	792,382

CENTERS OF EXCELLENCE/CENTERS OF EMPHASIS
ACTUAL, PROPOSED, AND REQUESTED BUDGET

Institution: Veterinary Medicine Center: Livestock Diseases and Human Health

	1997-98 Actual			1998-99 Proposed			1999-2000 Requested		
	Matching	Appropr.	Total	Matching	Appropr.	Total	Matching	Appropr.	Total
Expenditures	251,200	502,400	753,600	258,350	516,700	775,050	271,268	542,535	813,803
Salaries									
Faculty	34,133	68,266	102,399	37,424	74,848	112,272	39,295	78,591	117,886
Other Professional	36,781	73,561	110,342	44,227	88,453	132,680	46,438	92,876	139,314
Clerical/ Supporting	19,608	39,216	58,824	24,843	49,685	74,528	26,085	52,169	78,254
Assistantships	14,598	29,197	43,795	15,561	31,123	46,684	16,339	32,679	49,018
Total Salaries	105,120	210,240	315,360	122,055	244,109	366,164	128,157	256,315	384,472
Fringe Benefits	22,323	44,645	66,968	26,682	53,365	80,047	24,411	48,823	73,234
Total Personnel	127,443	254,885	382,328	148,737	297,474	446,211	152,568	305,138	457,706
Non-Personnel									
Travel	481	962	1,443	500	1,000	1,500	525	1,050	1,575
Software	450	900	1,350			0			0
Books & Journals	197	394	591			0			0
Other Supplies	45,455	90,912	136,367	41,826	83,653	125,479	43,918	87,835	131,753
Equipment	69,713	139,426	209,139	47,287	94,573	141,860	49,651	99,302	148,953
Maintenance	7,932	15,864	23,796	8,333	16,667	25,000	8,750	17,500	26,250
Scholarships	4,340	8,679	13,019	11,667	23,333	35,000	12,250	24,500	36,750
Consultants			0			0			0
Renovation			0			0			0
Other (Specify)			0			0			0
			0			0			0
			0			0			0
			0			0			0
Total Non-Personnel	128,568	257,137	385,705	109,613	219,226	328,839	115,094	230,187	345,281
GRAND TOTAL	256,011	512,022	768,033	258,350	516,700	775,050	267,662	535,325	802,987
Revenue									
New State Appropriation		502,400	502,400		516,700	516,700		535,325	535,325
Carryover State Appropriation		9,622	9,622			0			0
New Matching Funds	251,200		251,200	258,350		258,350	267,662		267,662
Carryover from Previous Matching Funds	4,811		4,811			0			0
Total Revenue	256,011	512,022	768,033	258,350	516,700	775,050	267,662	535,325	802,987

Schedule 7

CENTERS OF EXCELLENCE/CENTERS OF EMPHASIS
ACTUAL, PROPOSED, AND REQUESTED BUDGET

Institution: Veterinary Medicine Center: Livestock Diseases and Human Health

	1998-99 Actual			1999-2000 Proposed			2000-2001 Requested		
	Matching	Appropr.	Total	Matching	Appropr.	Total	Matching	Appropr.	Total
Expenditures									
Salaries									
Faculty	30,267	60,533	**90,800**	30,667	61,333	**92,000**	32,200	64,400	**96,600**
Other Professional	51,325	102,650	**153,975**	44,558	89,118	**133,676**	46,786	93,572	**140,358**
Clerical/ Supporting	19,173	38,346	**57,519**	17,188	34,376	**51,564**	18,047	36,095	**54,142**
Assistantships	17,717	35,435	**53,152**	26,146	52,291	**78,437**	27,453	54,907	**82,360**
Total Salaries	**118,482**	**236,964**	**355,446**	**118,559**	**237,118**	**355,677**	**124,486**	**248,974**	**373,460**
Longevity	1,696	3,393	**5,089**	1,377	2,754	**4,131**	1,446	2,892	**4,338**
Fringe Benefits	25,787	51,574	**77,361**	23,187	46,374	**69,561**	24,346	48,693	**73,039**
Total Staff Benefits	**27,483**	**54,967**	**82,450**	**24,564**	**49,128**	**73,692**	**25,792**	**51,585**	**77,377**
Total Personnel	**145,965**	**291,931**	**437,896**	**143,123**	**286,246**	**429,369**	**150,278**	**300,559**	**450,837**
Non-Personnel									
Travel	191	382	**573**			**0**			**0**
Software	468	936	**1,404**			**0**			**0**
Books & Journals	393	785	**1,178**			**0**			**0**
Other Supplies	34,574	69,147	**103,721**	52,427	104,854	**157,281**	55,048	110,097	**165,145**
Equipment	57,674	115,348	**173,022**	50,000	100,000	**150,000**	52,500	105,000	**157,500**
Maintenance	12,379	24,757	**37,136**	10,000	20,000	**30,000**	10,500	21,000	**31,500**
Scholarships	5,669	11,339	**17,008**	5,000	10,000	**15,000**	5,250	10,500	**15,750**
Consultants			**0**			**0**			**0**
Renovation			**0**			**0**			**0**
Other (Specify)			**0**			**0**			**0**
			0			**0**			**0**
			0			**0**			**0**
			0			**0**			**0**
Total Non-Personnel	**111,348**	**222,694**	**334,042**	**117,427**	**234,854**	**352,281**	**123,298**	**246,597**	**369,895**
GRAND TOTAL	**257,313**	**514,625**	**771,938**	**260,550**	**521,100**	**781,650**	**273,576**	**547,156**	**820,732**
Revenue									
New State Appropriation		518,900	**518,900**		521,100	**521,100**		547,156	**547,156**
Carryover State Appropriation		37,829	**37,829**		42,104	**42,104**			**0**
New Matching Funds	259,451		**259,451**	260,550		**260,550**	273,576		**273,576**
Carryover from Previous Matching Funds	18,194		**18,194**	21,052		**21,052**			**0**
Total Revenue	**277,645**	**556,729**	**834,374**	**281,602**	**563,204**	**844,806**	**273,576**	**547,156**	**820,732**

NOTE: FY 1999 carryover State Approp. of $37,827 was adjusted $2 to reflect overage.

CENTERS OF EXCELLENCE/CENTERS OF EMPHASIS
ACTUAL, PROPOSED, AND REQUESTED BUDGET

Institution College of Veterinary Medicine **Center** Livestock and Human Health

	1999-2000 Actual			2000-2001 Proposed			2001-2002 Requested		
	Matching	Appropr.	Total	Matching	Appropr.	Total	Matching	Appropr.	Total
Expenditures	260,550	521,100	781,650	265,250	530,500	795,750	278,082	557,456	835,538
Salaries									
Faculty	28,544	57,220	85,764	37,463	75,099	112,562	31,672	63,492	95,164
Other Professional	54,888	110,030	164,918	77,316	154,992	232,308	81,182	162,741	243,923
Clerical/ Supporting	12,303	24,662	36,965	9,762	19,570	29,332	10,250	20,548	30,799
Assistantships	18,255	36,595	54,850	20,015	40,122	60,137	21,015	42,128	63,144
Total Salaries	113,989	228,508	342,497	144,556	289,783	434,339	144,120	288,910	433,030
Longevity	1,327	2,660	3,987	1,331	2,669	4,000	1,398	2,802	4,200
Fringe Benefits	25,737	51,595	77,332	31,792	63,731	95,523	31,695	63,538	95,233
Total Personnel	141,054	282,762	423,816	177,679	356,183	533,862	177,213	355,250	532,463
Non-Personnel									
Travel	1,126	2,258	3,384	1,331	2,669	4,000	0	0	0
Software	0	0	0	0	0	0	0	0	0
Books & Journals	0	0	0	0	0	0	0	0	0
Other Supplies	41,627	83,446	125,073	50,112	100,457	150,569	34,333	68,824	103,157
Equipment	37,785	75,746	113,531	62,369	125,029	187,398	52,558	105,360	157,918
Maintenance	10,340	20,728	31,068	13,313	26,687	40,000	13,978	28,022	42,000
Scholarships	2,129	4,267	6,396	3,328	6,672	10,000	0	0	0
Consultants	0	0		0	0	0	0	0	0
Renovation	0	0	0	0	0	0	0	0	0
Other (Specify)	3,630	7,276	10,906	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0
Total Non-Personnel	96,636	193,722	290,358	130,454	261,513	391,967	100,869	202,206	303,075
GRAND TOTAL	237,690	476,484	714,174	308,133	617,696	925,829	278,082	557,456	835,538
Revenue									
New State Appropriation		521,100	521,100		530,500	530,500		557,025	557,025
Carryover State Appropriation		42,104	42,104		86,720	86,719			0
New Matching Funds	260,550		260,550	265,250		265,250	278,513		278,513
Carryover from Previous Matching Funds	21,050		21,050	43,360		43,360			0
Total Revenue	281,600	563,204	844,804	308,610	617,220	925,829	278,513	557,025	835,538